Proposed Panel Conclusions and Recommendations for the Bovine Corneal Opacity and Permeability Test Method

Expert Panel Meeting

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BCOP Test Method Primary Reviewers

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BRD Section 1.0:

BCOP Test Method Rationale

1.1 Scientific Basis

- **Mechanistic Basis -- Uses viable corneal tissue**

- **Endpoints**
  - **Opacity -- protocol doesn't differentiate mechanisms**
  - **Permeability**

- **Limitations**
  - **Only evaluates cornea**
  - **In the current form it will miss materials that do not cause grossly visible damage, i.e. mustard gas**
  - **Doesn't evaluate damage to limbal stem cells**
  - **Ignores protective mechanisms that operate *in vivo***

1.2 Regulatory Rationale and Applicability

Measures corneal changes similar to *in vivo* test

Unlike the *in vivo* rabbit eye test, BCOP does not assess iris, conjunctiva, including limbus, or systemic damage

BRD states BCOP cannot predict long term damage.

The document should discuss at a minimum

- **Work of Maurer & Jester providing evidence that initial changes can predict long term effects**
- **Human clinical experience with injury scales that are used to predict long term effects**

BRD Section 2.0:

BCOP Test Method Protocol Components

2.1 Components for Recommended Protocol (1)

Description of components is adequate

Eyes:

- From adult animals -- 18-48 months
- Antibiotics not effective at 4° C
- Storage time may be too restrictive
- BSE is a risk

Solvent for preparing solutions:

- Use 0.9% NaCl -- not sterile water
- Osmolarity and pH of solutions should be known

2.1 Components for Recommended Protocol (2)

Corneal culture medium

- MEM with FBS not necessary
- Balanced salt solutions should be acceptable

Optimize corneal holder

- Clamp on sclera not cornea
- Maintain curvature of cornea
- Prevent crush injury to cornea

Exposure

- Optimize exposure duration for ‘volatile solvents’
- Exposure method for solids is problematic

2.1 Components for Recommended Protocol (3)

Optimize rinsing procedures

Histopathology must be added unless

the substance is from a class of materials known to be accurately predicted using only opacity and permeability in the BCOP assay

A grading system for histopathology is needed

Identification of reference substances that are part of the performance standards developed for the validated test method

2.1 Components for Recommended Protocol (4)

Controls Needed

- Positive, negative and benchmark controls are needed
- Each laboratory must establish acceptable ranges

Reexamine Prediction Model

- Is a calculated score advisable/necessary?
- Optimize to identify severe irritants
- The BRD should identify the decision criteria (Prediction Model) for identifying ocular corrosives and severe irritants and discuss rationale for development

2.2 Basis for Selection of the Test Method System

- The panel believes the BRD discussion and evaluation are appropriate.

2.3 Proprietary Components

- None. Specifications for the corneal holder and the opacitometer should be included in the recommended protocol

2.4 Number of Replicate and/or Repeat Experiments for Each Test

- The panel believes the BRD discussion and evaluation are appropriate.

2.5 Study Acceptance Criteria

- The panel believes the BRD discussion and evaluation are appropriate.

2.6 Basis for any Modifications to the Original Test Method Protocol

- The Panel believes the BRD discussion is appropriate.

2.7 Adequacy of the Recommended Protocol

- **Critical changes are standardized age of cattle, increased consideration of BSE risk, replacement of distilled water with 0.9% NaCl, addition of histopathology, and optimization of the test for alcohols, ketones and solids**

- **Other proposed changes, in particular the suggested holder, could improve the test by reducing variability and should be investigated as part of a continuing effort to optimize the test**

BRD Section 3.0:

Substances Used for Previous Validation Studies of the BCOP Test Method

3.1 Types & Numbers of Substances Used for Prior Validation Studies

The number and classes of substances were acceptable

Materials known to be severe eye irritants in humans should be confirmed to be positive in BCOP

Since available data indicates alcohols, ketones and solids are problematic in BCOP, better chemical characterization and physicochemical data on all of the test substances are needed

3.2 Coding Procedures for Test Substances and Quality of BCOP Test Method Data

- Panel considers coding to be important; if not used, data quality could be affected. Coding procedures were considered adequate.

BRD Section 4.0:

In Vivo Reference Data Used for an Assessment of Test Method Accuracy

4.1 *In Vivo* Rabbit Eye Test Method Protocols Used to Generate Reference Data

The *in vivo* rabbit eye test method protocols used to generate reference data in the cited studies were appropriate

4.2 Interpretation of *In Vivo* Test Method Results for Cited Studies

The use of the three regulatory classification systems to evaluate *in vitro* methods is questioned

4.3 Data Quality for Test Substances

The lack of original study records is recognized but not considered serious enough to prevent use of the data

4.4 Data Quality With Respect to Extent of GLP Compliance

BRD should include more information on GLP compliance of the *in vivo* studies

4.5 Availability of Relevant Human Ocular Toxicity Information

- **Confirm current ocular hazard classification schemes are adequate by examining Poison Control Center Data, Dept. of Labor data and reviewing published case reports.**
- **There needs to be greater effort to obtain and consider information on human topical ocular chemical injury.**

4.6 Accuracy and Reliability of the *In Vivo* Rabbit Eye Test

- The potential variability of the rabbit eye data has not been adequately discussed
- Discussion should include Weil and Scala (1971), Haseman (2005) and Kaneko (1999) data.
- Attempt to confirm *in vivo* classifications using other data sources such as RTECS or IUCLID
- Any optimization and validation studies should use existing animal data; if available.
- Additional animal studies should only be conducted if important data gaps are identified and such studies should be carefully designed to maximize the amount of pathophysiological information obtained (e.g., wound healing).
- Minority opinion – no animal testing for this purpose.

BRD Section 5.0:

BCOP Test Method Data and Results

5.1 BCOP Test Method Protocols Used to Generate Each Set of Data

- The Panel agrees with the BRD assessment of these data.

5.2 Other Comparative BCOP - *In Vivo* Rabbit Eye Test Data Not Considered in the BRD

- The Panel is not aware of other data that include raw scores for both tests.

5.3 Statistical and Nonstatistical Approaches Used to Evaluate the Resulting BCOP Data

- The statistical methods used to assess the data seem appropriate.

5.4 Use of Coded Substances, Blind Studies, and GLP Guidelines for Cited Studies

The Panel agrees with the BRD assessment of this information.

The lack of GLP compliance should not *a priori* exclude data from evaluation.

5.5 "Lot-to-Lot" Consistency of Test Substances and Timeframe of Studies

The Panel agrees with the BRD assessment of this information.

BRD Section 6.0:

BCOP Test Method Accuracy

a. The closeness of agreement between a test method result and an accepted reference value.

b. The proportion of correct outcomes of a test method

6.1 Accuracy Evaluation of the BCOP Test Method for Identifying Ocular Corrosives and Severe Irritants as Defined by the EPA (1996), the EU (2001), and the GHS (2003)

The BCOP as it is currently run, with histopathology, is acceptable to assess the ability of materials to cause corrosive or serious injury to the eye as part of the screening procedure described in the BRD.

Based on the data presented, the assessment of alcohols, ketones and solids with the protocol as written is problematic.

Accuracy parameters must indicate these are a concordance comparison with the results of a single rabbit eye test.

	No.	False Neg.		False Pos.	Accuracy
All GHS	120	24%		19%	79%
Without Alcohols & Ketones	99	22.5%		12%	84%
Solids	19	33%		29%	68%
Without Alcohols Ketones Solids	80	18%		10%	87.5%

BCOP Test Method

6.2 Strengths & Limitations of the Test Method, Including Those Applicable to Specific Chemical Classes or to Certain Physicochemical Properties

Based on the data presented, the assessment of alcohols, ketones and solids with the protocol as written is problematic.

Effect of colored substances not discussed

Consideration should be given to exploring physicochemical effects using a structure activity or structure property relationship program.

In addition to the analyses conducted, the Panel suggests an assessment based on ranking of experimental data for severity for both the reference method and the in vitro test

BRD Section 7.0:

BCOP Test Method Reliability (Repeatability/Reproducibility)

A measure of the degree to which a test method can be performed reproducibly within and among laboratories over time.

7.1 Selection Rationale for the Substances Used to Evaluate Test Method Reliability

The Panel agrees with the BRD assessment.

7.2 Analyses & Conclusions Regarding Intralaboratory Repeatability and Intra- & Inter-laboratory Reproducibility

The data from existing studies is extensively reviewed and considered in the document. The panel agrees that the data indicates acceptable levels of intra- and inter-laboratory variability.

Variability may be decreased if the protocol is optimized further.

CV's should be used with care with this data. The median CV may not be informative.

7.3 Availability of Historical Negative & Positive Control Data

Positive data are presented, negative control data are not available.

7.4 Effect of Minor Protocol Changes to Recommended Test Method Protocol and Transferability of Test Method

The data indicate the test is transferable. At what point 'minor' protocol changes will be sufficiently significant to require further validation cannot be determined with the information provided.

BRD Section 8.0:

BCOP Test Method
Data Quality

8.1 Extent of Adherence to GLP Guidelines and Use of Coded Chemicals

Coding should be used for all subsequent studies.

8.2 Data Quality Audit

Spot checks of data not part of multilaboratory validation studies could be conducted. The panel does not beieve this is necessary.

8.3 Impact of Deviations from GLP Guidelines

The Panel agrees with the BRD assessment of these data.

8.4 Availability of Laboratory Notebooks or Other Records for an Independent Audit

The lack of original notebook data is of some concern but not sufficient to remove the data from consideration. Recent information indicates that raw data may be available for many if not all of the studies included in this evaluation.

BRD Section 9.0:

Other Scientific Reports and Reviews

9.1 Adequacy and Completeness of Relevant Data Identified in Other Published or Unpublished BCOP Studies

Relevant data appear to be identified.

9.2 Adequacy and Completeness of the Conclusions Published in Independent Peer Reviewed Reports or Other Independent Scientific Reviews

The Panel agrees with the BRD assessment of these data.

9.3 Approaches that can be Used to Expedite the Process for Obtaining Additional In-House Data from the Private Sector

It is possible that more data could be obtained by working with trade associations, but much of the data in the BRD comes from these sorts of efforts, so whether more data could be obtained is unclear.

BRD Section 10.0:

Animal Welfare Considerations (Refinement, Reduction, Replacement)

10.1 Extent to Which the Test Method Will Refine, Reduce or Replace Animal Use

BCOP will reduce the numbers of animals exposed to severe irritants

The BCOP will classifying some substances without further animal tests

BRD Section 11.0:

Practical Considerations

11.1 Adequacy and Completeness of Test Method Transferability

- The BRD adequately addresses the facilities, major fixed equipment, and availability of other supplies needed to conduct the BCOP method.
- A training video and other visual media on the technical aspects of the assay is recommended (place in all)
- Training approaches in the application of this test method should be developed/implemented (place in all)

11.2 Adequacy and Completeness of Test Method Training

The required level of training and expertise to conduct BCOP are adequately considered.

The description of training of technicians for the *in vivo* test may be incorrect -- proficiency in the *in vivo* test is demonstrated the same way as for BCOP

11.3 Adequacy and Completeness of Test Method Cost

The discussion should be modified to reflect the public comments

11.4 Adequacy and Completeness of Amount of Time Needed to Conduct Test Method

For very corrosive substances and some severe irritants, the evaluation may be completed within 4 hours in the *in vivo* test.

BRD Section 12.0:

Proposed BCOP Test Method Recommendations

12.1 Recommended Version of the BCOP Test Method

Confirm with several active laboratories that proposed changes are workable

12.2 Recommended Standardized BCOP Test Method Protocol (1)

For the purpose of detecting severe eye irritants in the testing scheme outlined in the BRD, the BCOP test presented is useful in identifying ocular corrosives and severe irritants, as described in the BRD, with the following exception of

- **Alcohols, ketones, and solids are problematic**

Histopathological examination must be added, unless the substance is from a class of materials known to be accurately predicted using only opacity and permeability in the BCOP assay

12.2 Recommended Standardized BCOP Test Method Protocol (2)

- Confirm BCOP test identifies substances known to cause serious eye injury in humans
- Negative, positive and benchmark controls are added
- Use eyes from young adult cattle
- Users should be aware of the risk of BSE and other zoonoses and use proper precautions
- Use 0.9% NaCl as standard diluent and rinse
- Determine osmolarity and pH of test solutions

12.3 Recommended BCOP Optimization Studies

Recommended future improvements

- Larger holder designed by Ubels
- Reexamining the calculated total score
- Optimize media used to bathe the eyes
- Optimize rinsing procedures
- Consider use of younger animals
- Discourage the use of antibiotics

Optimization studies will be necessary to ensure any changes to the protocol will decrease the variability of the test method

12.3 Recommended BCOP Validation Studies (1)

Protocol for solids

- **improved exposure methods**

Protocol for alcohols and ketones

- **3 minute exposure time**

May be satisfied by the submission of additional historic data

Validation is not required for the addition of histopathology or changes in scoring system

12.3 Recommended BCOP Validation Studies (2)

- Any optimization and validation studies should use existing animal data; if available.
- Additional animal studies should only be conducted if important data gaps are identified and such studies should be carefully designed to maximize the amount of pathophysiological information obtained (e.g., wound healing)
- Minority opinion –sufficient data should be available so no animal testing for this purpose – Dr. Stephens
- Reference substances should be identified that can be used as part of performance standards
- NICEATM/ICCVAM should facilitate the development of a histopathology scoring system for corneal damage (with visual aids)

Additional Comment

Consider a protocol using porcine eyes

Thank you